REMARKS

Please amend the application as indicated prior to consideration of this application on the merits. Applicants have filed this continuation application to continue prosecution of claims in the parent application U.S. Serial No. 09/107,051, (the "parent application"). Please amend claims 1 and 7 to 11 and cancel claims 12-20. Applicants reserve the right to pursue the subject matter of the cancelled claims[1] in a separate application.

The specification is merely amended to include a specific reference to the prior application. Claim 1 is amended simply to further define the invention. The amendment of claim 1 is supported by the specification at, for example, page 23, lines 13-16. Claims 7-11 are amended for the sake of consistency.

No new matter would be added by the proposed amendment.

The Invention

Applicants have discovered that bone marrow stromal cells (BMSCs) that have been transfected with an exogenous gene and then cryopreserved express the exogenous gene at a level that is comparable to that seen prior to cryopreservation. This method is now claimed in pending claims 1-11.

35 U.S.C. § 103(a)

Claims 1-5, 7-9, 12-14, 16-18, and 20 were rejected in the parent application as being unpatentable over three references: Greenberger *et al.* (EP 0 381 490; herein, "Greenberger"), Boswell *et al.* (*Exp. Hematol.* 11:315-323, 1983; herein, "Boswell"), and Motta *et al.* (*Bone Marrow Transp.* 12:177, 1993; herein, "Motta").

Applicants have cancelled claims 12-20. Thus, the rejection of claims 12-14, 16-18, and 20 is now moot. In addition, Applicants have amended claim 1. In view of this amendment and the remarks that follow, this ground for rejection should not be repeated in this continuation application.

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[1] These claims covered methods in which bone marrow stromal cells are cryopreserved at a different point in the procedure (*e.g.*, before transfection or before being cultured) than the point claimed in claims 1-11.

In the parent application the the Examiner described the three references cited against the application as follows. Greenberger is characterized as disclosing "a method of gene therapy using genetically modified bone marrow stromal cells" (Final Office Action in parent application at page 2); Boswell is characterized as disclosing that "stromal progenitor cells [are] capable of transferring the hematopoietic microenvironment to liquid culture" (Office Action at page 3); and Motta is characterized as disclosing the successful use of cryopreserved bone marrow cells "after ten years of cryopreservation" (Office Action at page 3).

The Examiner then concluded that "it would have been obvious ... to modify the methods of Greenberger by incorporating cryopreservation of the cultured stromal cells as taught by Boswell or Motta" (Office Action at page 3). The Examiner stated that the required motivation would come from a desire to "avoid steps, time and labor to make the cells again" (Office Action at page 3), and that the required expectation of success would come from "the results of Boswell and Motta," who allegedly showed that "cryopreservation and thawing of marrow would still reproduce the bone marrow microenvironment *in vitro*" (Office Action at page 3).

<u>There is No Motivation to Combine Greenberger with Boswell and Motta</u>

Obviousness can only be established where there is some teaching, suggestion, or motivation to combine or modify the teachings of the prior art. The requisite motivation can be found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. MPEP at 2143.01.

As noted above, the Examiner suggested in the parent application that one of ordinary skill in the art would have modified the methods of Greenberger by incorporating cryopreservation to "avoid steps, time and labor to make the cells again" (Office Action at page 3). However, the Office Action provided no evidence or reasoning to support this statement. More importantly, nothing in these references describes or suggests cryopreservation of <u>transfected</u> BMSCs. Boswell and Motta both cryopreserve bone marrow, which is a complex mixture of cells containing very few BMSCs (BMSCs constitute only about 0.1% of bone marrow). Nothing in Boswell or Motta suggests cryopreservation of transfected BMSCs. Moreover, nothing in the prior art or in the knowledge generally available to those of ordinary skill in the art suggests that cryopreservation should be carried out *following* BMSC transfection,

as required by the present claims. One cannot arrive at the present invention by incorporating cryopreservation at just any point in a transfection procedure, and there is certainly no suggestion to incorporate it, as applicants have, following BMSC transfection. On this basis alone, the rejection for obviousness should not be applied to amended claim 1.

Greenberger, Boswell, and Motta do Not Suggest all of the Present Claim Limitations

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. MPEP at 2143.03.

Even if combined, Greenberger, Boswell, and Motta cannot render obvious the method of claim 1 because nothing in these references suggests preparation of BMSCs that express comparable levels of an exogenous gene before and after cryopreservation. As the Examiner admitted in the parent application, "Greenberger et al. do not teach cryopreservation of stromal cells" (Office Action at page 2), and neither Boswell nor Motta preserved transfected cells. Thus, none of the prior art can suggest a method of obtaining a preparation of BMSCs that requires comparable exogenous gene expression before and after cryopreservation. Given this failure, claim 1 should not be rejected.

Greenberger, Boswell, and Motta Fail to Provide the Requisite Expectation of Success

Another requirement for a *prima facie* case of obviousness is that there must be a reasonable expectation of success. MPEP at 2143. The courts have long held that the prior art must not only suggest that something may be tried, but also that the attempt would have a reasonable likelihood of success. *In re Dow Chemical Co.,* 837 F.2d 469, 473 (Fed. Cir. 1988). It is not enough that the prior art render an invention obvious to try. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 803 F.2d 1367 (Fed. Cir. 1986) (the district court erred in invalidating the patent on the ground that it was "obvious-to-try").

In the parent case, the Examiner argued that a reasonable expectation of success would come from "the results of Boswell and Motta," which allegedly showed that "cryopreservation and thawing of marrow would still reproduce the bone marrow microenvironment *in vitro*" (Office Action at page 3).

While the Examiner is correct in noting that Boswell and Motta worked with cryopreserved marrow, neither can provide the requisite expectation for success for the method presently claimed. Neither Boswell nor Motta worked with transfected cells, much less transfected BMSC. Thus, neither Boswell nor Motta can shed any light on whether or to what extent cryopreserved BMSCs would express an exogenous gene, let alone whether they would do so to an extent comparable to that seen in BMSCs that have not been cryopreserved (see amended claim 1). Indeed, Motta's relevance to Applicants' claim 1 is quite limited. Motta reports a successful case of hemopoietic reconstitution in a patient (*i.e.*, n = 1) who received a complex mixture of autologous bone marrow cells that were not transfected (as required by the present method). Those of ordinary skill in the art would not have read Motta's anecdotal report as a predictor of success with Applicants' method, particularly given that Motta failed to use (indeed, did not mention) transfected cells. There would have been no way to predict, given Motta's distinct population of non-transfected cells, that applicants' transfected BMSCs could be cryopreserved so that they express an exogenous gene at a level comparable to that in non-cryopreserved cells. Motta's experiments support a single conclusion -- that a complex mixture of non-transfected bone marrow cells can be transplanted despite a long storage period -- and have no predictive value whatsoever for applicants' claimed method.

Boswell similarly fails to disclose BMSCs that have been transfected. Moreover, in attempting to design a new assay for the recovery of stromal cells, Boswell found that even "normal" stromal cells suffered when cryopreserved. For example, Boswell reports that (page 318; emphasis added):

> [w]hen an empiric system of stromal grading was applied to the ... appearance of the stromal lawn ... it was apparent that the frozen group was retarded in extent of stromal development.

Although Boswell's cryopreserved cells later appeared to be comparable to control (*i.e.*, to cells that had not been cryopreserved), it took the cryopreserved cells seven weeks in culture to catch up (see Figure 2). If cryopreserved cells were "retarded" in their ability to establish a stromal layer, one can only expect that cryopreserved cells that are also transfected, as required by applicants' claim 1, would be retarded to at least the same extent (and perhaps more, given that transfection necessarily disrupts the cells' plasma membranes). Indeed, given Boswell's

observation, what one would reasonably expect is that cryopreserved cells that were "retarded" in their ability to establish a stromal layer, would also be retarded in other ways, *e.g.,* in their ability to express an exogenous gene. Certainly, nothing in Boswell would have provided a reasonable basis to expect that transfected BMSCs could be successfully cryopreserved, much less that those cells would express an exogenous gene at levels comparable to those seen in cells that have not been cryopreserved. Accordingly, this ground for rejection should not be repeated in the present application.

Dependent Claim Rejections

Claims 10 and 11 were rejected in the parent case under 35 U.S.C. § 103(a) as being allegedly unpatentable over Greenberger, Boswell, and Motta and further in view of Lobb *et al.* (*Biochem. Biophysic. Res. Comm.* 178:1598-1504, 1991; herein, "Lobb"). The Examiner characterized Lobb as disclosing the expression of a vascular cell adhesion molecule (VCAM1). The Examiner stated that Lobb discloses "that VCAM1 selectively binds to CD8+ memory T cells and should prove useful for immune responses *in vivo*" (Office Action at page 4). This ground for rejection should not be repeated here given the amendment of claim 1, from which claims 10 and 11 depend or ultimately depend.

For the reasons described above, claim 1 would not have been obvious in view of Greenberger, Boswell, and Motta. Since claims 10 and 11 incorporate all of the limitations of claim 1, and since Lobb does nothing to suggest those limitations, claims 10 and 11 cannot be obvious. This ground for rejection should therefore not be repeated here.

Claims 6, 15, and 19 were rejected in the parent application under 35 U.S.C. § 103(a) as being allegedly unpatentable over Lozier *et al.* (*Human Gene Therapy* 5:313-322, 1994; herein, "Lozier") in view of Boswell and Motta. In the parent application the Examiner characterized Lozier as disclosing the preparation of BMSCs from canines with hemophilia B and the transfection of these cells with canine Factor IX (Office Action at page 5).

Claims 15 and 19 have been cancelled. Thus, the rejection of these claims is now moot. With respect to claim 6, the rejection should not be repeated here given the amendment of claim 1, from which claim 6 depends.

For the reasons described above, claim 6 would not have been obvious in view of Greenberger, Boswell, and Motta. Lozier, which focuses on canine cells, can do no more than Greenberger when combined with Boswell and Motta. Moreover, since claim 6 incorporates all of the limitations of claim 1, and since Lozier does nothing to suggest those limitations, claim 6 cannot be obvious. This ground for rejection should not be repeated here.

CONCLUSION

Applicants submit that all of the claims are now in condition for allowance, which action is requested. No fees are believed due in connection with this response. If there are any fees, or any credits, please apply them to Deposit Account No. 06-1050, referencing Attorney Docket No. 07787-004003.

Respectfully submitted,

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